January 7, 2002

**RETURN RECEIPT REQUESTED, PLEASE, BY E-MAIL**

Christine Todd Whitman, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116

ATTN: Chemical Right-to-Know Program

**RE: Submission of test plan pursuant to the High Production Volume challenge for Acetamide, N-[5-[bis[2-(acetyloxy)ethyl]amino]-2-[(2-bromo-4,6-dinitrophenyl)azo]-4-methoxyphenyl]- (i.e., C.I. Disperse Blue 79:1) CAS No. 3618-72-2**

Dear Administrator Whitman:

The ETAD North America Disperse Blue 79:1 consortium (formerly USOC/ETAD Disperse Blue 79:1 consortium) is pleased to submit the test plan and robust summary in IUCLID format for Acetamide, N-{5-(bis[2-acetyloxy)ethyl]amino]-2-[(2-bromo-4,6-dinitrophenyl)azo]-4-methoxyphenyl]- (i.e., C.I. Disperse blue 79:1), CAS No. 3618-72-2.

Sponsoring companies who are members of this consortium are:

Blackman Uhler Chemical Company
Ciba Specialty Chemicals Corporation
Clariant Corporation
DyStar L.P.

ETAD North America represents the interests of dye manufacturers and formulators in the NAFTA Region. Its parent organization, the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (ETAD), is an international technical organization that addresses the health, environmental, and safety aspects of the worldwide colorants manufacturing industry.

The undersigned is technical contact for all matters pertaining to this HPV submission. He can be reached at 202-721-4154 or by e-mail at tucker.helmes@socma.com. Please note, after January 14, 2002, the correct e-mail address will be helmest@socma.com.

Sincerely,

C. Tucker Helmes, Ph.D.
Executive Director

AR201-13461A



**Table 1. Test Plan for C.I. Disperse Blue 79:1 (CAS No. 3618-72-2)**

| Endpoint | Data Available | Acceptable | Planned Testing |
|---|---|---|---|
| | ***Physical/Chemical Elements*** | | |
| Melting Point | >/= 138° C | Yes | |
| Boiling Point | 476° C (1) | Yes | |
| Vapor Pressure | $4.53X10^{-9}$ hPa @ 25° C (1) | Yes | |
| Partition coefficient | 4.44@ 25°C | Yes | |
| Water solubility | 5.2 µg/l @ 25°C | Yes | |
| | ***Environmental Fate and Pathways Elements*** | | |
| Photodegredation | $T_{1/2}$ = 0.568 hr (EPIWIN) | Yes | |
| Stability in water | $T_{1/2}$ = </= 4 hr | Yes | |
| Fugacity | Sediment sorption | Yes | |
| Biodegredation | Anaerobic degredation | Yes | |
| | ***Ecotoxicity Elements*** | | |
| Acute Toxicity/fish | NOEC > 4.8 µg/l | Yes | |
| Toxicity/aquatic plants | Algae (1) | Yes | |
| Acute toxicity/ aquatic invertebrates | Daphnia (1) | Yes | |
| | ***Health Elements*** | | |
| Acute toxicity | MTD=2500 mg/kg/day | Yes | |
| Mutagenicity *in vitro* | Positive (Ames) | Yes | |
| Mutagenitcity *in vivo* | Negative (fruit fly) | Yes | |
| Repeat dose toxicity | NOAEL > 2500 mg/kg/day | Yes | |
| Reproductive toxicity | NOAEL > 2000 mg/kg/d (rat) | Yes | |
| | NOAEL > 100 mg/kg/d (rab) | Yes | |
| Teratogenicity | NOAEL > 2000 mg/kg/d (rat) | Yes | |
| | NOAEL > 300 mg/kg/day (rab) | Yes | |

Notes

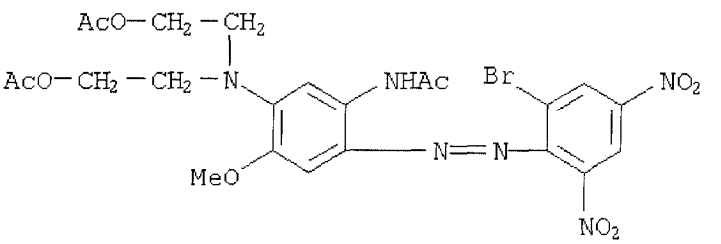
(1) Reported data are for related dye, C.I. Disperse Blue 79 (CAS No. 12239-34-8)

**C.I. Disperse Blue 79:1**
CAS No. 3618-72-2

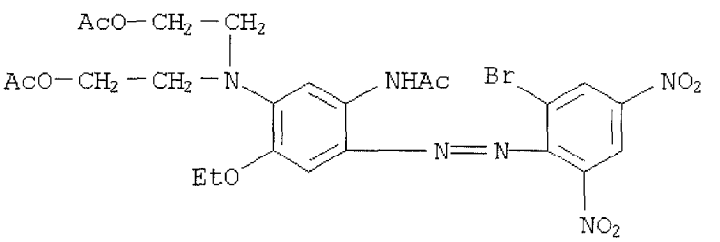
**Test Plan Justification**

C.I. Disperse Blue 79:1 (DB79:1) has been tested extensively as documented in the accompanying IUCLID robust summary and attached test plan. As a result of a voluntary test program conducted by U.S. dye makers, the EPA in 1993 declared that sufficient test data exist for DB79:1 to indicate relatively low toxicity and low concern for environmental risk, and that further work on DB79:1 was not justified (1). In fact, the agency removed DB79:1 from its Master Testing List in 1992 because it had received, reviewed, and accepted the results of all tests required under TSCA Section 4 (2).

In the few instances shown below where test data do not exist for DB79:1 (e.g., vapor pressure, toxicity/algae), data are provided for the close structural analog, C.I. Disperse Blue 79 (CAS No. 12239-34-8). The justification for using data on Disperse Blue 79 as surrogate data for DB79:1 is evident from examination of the similarity of their structures:




**C.I. Disperse Blue 79:1**. Chemical name: Acetamide, N-[5-[bis[2-(acetyloxy)ethyl]amino]-2-[(2-bromo-4,6-dinitrophenyl)azo]-4-methoxyphenyl]-




**C.I. Disperse Blue 79.** Chemical name: Acetamide, N-[5-[bis[2-(acetyloxy)ethyl]amino]-2-[(2-bromo-4,6-dinitrophenyl)azo]-4-ethoxyphenyl]-

The structures of these two related dyes are identical in every respect except for the 4-methoxyphenyl moiety in DB79:1 which is a 4-ethoxyphenyl moiety in Disperse Blue 79. One would anticipate that this slight change in chemical structure would have very little impact on the chemical, physical, and biological properties. In fact, the partition

coefficients of the two substances are virtually identical. For DB79:1, Log $P_{ow}$ = 4.44, as documented in the attached test plan and robust summary, while for Disperse Blue 79 Log $P_{ow}$ = 4.1 (3). Similarly, the water solubility of DB79:1 is 5.2 μg/l @ 25° C and for Disperse Blue 79 it is 5.4 μg/l @ 25° C (3).

Further similarities between the two structures are demonstrated by the EPIWIN modeling program recommended by the HPV Challenge Guidance. EPIWIN predicts an overall hydroxyl radical photodegradation rate constant for DB79:1 of 2.26 X $10^{-10}$ $cm^3$/molecule-sec, with a half-life of 0.568 hours at hydroxyl concentration of 1.5 X $10^6$ molecules/$cm^3$. This compares to the predicted values for Disperse Blue 79 of a rate constant of 1.49 X $10^{-10}$ $cm^3$/molecule-sec, with a half-life of 0.863 hours at hydroxyl concentration of 1.5 X $10^6$ molecules/$cm^3$ (4).

***Physical/Chemical Elements***. The physical/chemical properties of DB79:1 that are documented in the attached robust summary were obtained from the published scientific literature or manufacturer's material safety data sheet (MSDS). All reported data appear reliable and are based on standard methodology, so no additional testing is planned.

***Environmental Fate and Pathways Elements.*** Valid experimental data on DB79:1 are reported in the attached robust summary for three of the four required HPV end points: stability in water, fugacity, and biodegradation. For photodegradation, the fourth required end point, data were modeled using the EPIWIN program, as recommended by the HPV Challenge Guidance.

DB79:1 is removed from effluent by the settling of particulate matter and adsorption on activated sludge. Complete removal of DB79:1 occurs through anaerobic degradation. No dye is found in sediment or water samples downstream from the wastewater treatment plant.

No data gaps exist requiring further testing.

***Ecotoxicity Elements.*** Test results of a valid and very thorough acute fish toxicity test of DB79:1 are summarized in the attached robust summary and test plan. In this study, no toxicity of the dye was observed at concentrations up to the experimental limits of water solubility, > 4.8 μg/l. No data are available on DB79:1 specifically for toxicity in aquatic plants or aquatic invertebrates, but data are summarized for these two end points on the close structural relative, Disperse Blue 79. As explained above, these data are acceptable surrogates for DB79:1. Therefore, no additional testing is planned.

***Health Elements.*** All HPV-required health endpoints for DB79:1 have been fulfilled satisfactorily by the results of previous studies conducted voluntarily by U.S. dye makers. These studies are documented in the attached robust summary and test plan.

DB79:1 is not toxic in rats by oral administration when administered in a 14-day acute study or in a 90-day repeated dose study at daily doses up to 2,500 mg/kg/bw. Although found to be positive in the Ames *Salmonella* bacterial mutagenicity assay, subsequent tests for genetic toxicity were negative in mammalian V79 cells, the mouse micronucleus assay, and the *Drosophila* (fruit fly) SLRL mutagenicity test.

Similarly, no reproductive toxicity or teratogenicity was observed in rats at doses up to 2,000 mg/kg/bw. In rabbits, some maternal toxicity and fetal body weight reduction were

observed at 300 and 600 mg/kg/bw, respectively, but there was no evidence of teratogenicity at any dose tested up to 600 mg/kg/bw.

Additional studies on the metabolism of DB79:1 in rats indicated that the dye is not extensively absorbed from the GI tract but is substantially cleared without undergoing any significant metabolism.

No further testing is necessary to satisfy the health-related endpoints for the HPV Challenge.

## REFERENCES


1. EPA (January 11, 1993). Letter with attachments from C. Auer, U.S. Environmental Protection Agency, to Dr. C.T. Helmes, ETAD.
2. Master Testing List (December 1, 1992). Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, DC.
3. Clariant (June 1996). IUCLID Dataset for C.I. Disperse Blue 79 (CAS No. 12239-34-8).
4. Meylan, W. and Howard, P. (2000). EPIWIN Modeling Program, Syracuse Research Corporation, Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510

# I U C L I D
# Data Set

| | |
|---|---|
| **Existing Chemical ID:** | 3618-72-2 |
| **CAS No.** | 3618-72-2 |
| **Product name** | C.I. Disperse Blue 79:1 |
| **Colour index number** | 11344 |
| **CAS Name** | Acetamide, N-[5-[bis[2-(acetyloxy)ethyl]amino]-2-[(2-bromo-4,6-dinitrophenyl)azo]-4-methoxyphenyl]- |
| **EINECS No.** | 222-813-1 |
| **Molecular Formula** | C23H25BrN6O10 |
| **Creation date:** | 27-FEB-2001 |
| **Consortium:** | ETAD North America Disperse Blue 79:1 Coalition |
| **Number of Pages:** | 20 |

**Company Information**

## 1.0.1 OECD and Company Information

| | |
|---|---|
| **Type:** | lead organization |
| **Name:** | ETAD North America Disperse Blue 79:1 Coalition |
| **Street:** | 1850 M Street, NW, Suite 700 |
| **City/State:** | Washington, DC |
| **Zip Code:** | 20036 |
| **Country:** | United States |
| **Phone:** | 202-721-4100 |
| **Telefax:** | 202-296-8120 |
| **Remark:** | Dr. C. T. Helmes - contact |

| | |
|---|---|
| **Type:** | cooperating company |
| **Name:** | Blackman Uhler Chemical Company |
| **Street:** | PO Box 5627 |
| **City/State:** | Spartanburg, SC |
| **Zip Code:** | 29304 |
| **Country:** | United States |
| **Phone:** | 864-585-3661 |
| **Telefax:** | 864-596-1501 |
| **Remark:** | Ron Matthews - Contact |

| | |
|---|---|
| **Type:** | cooperating company |
| **Name:** | Ciba Specialty Chemicals Corporation |
| **Street:** | PO Box 2444 |
| **City/State:** | High Point NC |
| **Zip Code:** | 27261-2493 |
| **Country:** | United States |
| **Phone:** | 336-801-2618 |

| | |
|---|---|
| **Telefax:** | 336-801-3077 |
| **Remark:** | Tom Dukes - Contact |
| | |
| **Type:** | cooperating company |
| **Name:** | Clariant Corporation |
| **Street:** | 4000 Monroe Road |
| **City/State:** | Charlotte NC |
| **Zip Code:** | 28205 |
| **Country:** | United States |
| **Phone:** | 704-331-7711 |
| **Telefax:** | 704-331-7718 |
| **Remark:** | Blair Drum - contact |
| | |
| **Type:** | cooperating company |
| **Name:** | DyStar L.P. |
| **Street:** | 9844-A Southern Pine Boulevard |
| **City/State:** | Charlotte NC |
| **Zip Code:** | 28273 |
| **Country:** | United States |
| **Phone:** | 704-561-2644 |
| **Telefax:** | 704-561-3098 |
| **Remark:** | Will Caylor - contact |

## PHYSICAL CHEMICAL PROPERTIES

### 2.1 Melting Point

| | |
|---|---|
| **Value:** | >= 138 degree C |
| **Year:** | 1989 |
| **GLP:** | no data |
| **Test substance:** | Foron Navy S-2GRL Purified Presscake (i.e., C.I. Disperse Blue 79:1) |
| **Reliability:** | (2) valid with restrictions |
| **Flag:** | Critical study for SIDS endpoint |
| **Reference:** | (9) |

### 2.2 Boiling Point

| | |
|---|---|
| **Value:** | = 476 degree C |
| **Year:** | 1996 |
| **GLP:** | no data |
| **Test substance:** | C.I. Disperse Blue 79 (CAS No. 12239-34-8) |
| **Reliability:** | (2) valid with restrictions |
| **Flag:** | Critical study for SIDS endpoint |
| **Reference:** | (16) |

### 2.4 Vapour Pressure

| | |
|---|---|
| **Value:** | = 4.53 x $10^{-9}$ hPa at 25 degree C |
| **Year:** | 1996 |
| **GLP:** | no data |
| **Test substance:** | C.I. Disperse Blue 79 (CAS No. 12239-34-8) |
| **Reliability:** | (2) valid with restrictions |
| **Flag:** | Critical study for SIDS endpoint |
| **Reference:** | (16) |

## 2.5 Partition Coefficient

**log Pow:** = 4.44 at 25 degree C
**method:** measured
**Year:** 1999
**GLP:** no data
**Method:** Disperse Blue 79:1 was first recrystallized in dichloromethane to remove any additives prior to use. In a 1-L thermostated flask, 800 ml of distilled water and 100 ml of octanol were added and slowly stirred with 0.4 g/L test substance at 25 degrees Celsius. The dye was dissolved in octanol, then the required amount of octanol was pipetted off and flushed gently into the thermostated flask on top of the water. During a three week period, several samples of water and octanol were taken and the concentration of the dye was determined in both solutions. The Kow was determined from the ratio of the concentrations in octanol and water, respectively, at equilibrium. Each measurement was performed in triplicate thermostated flasks.

**Test substance:** C.I Disperse Blue 79:1 was first recrystallized in dichloromethane to remove additives prior to use.

**Reliability:** (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment.

**Flag:** Critical study for SIDS endpoint

**Reference:** (10)

## 2.6.1 Water Solubility

**Value:** = 0.0052 mg/1 at 25 degree C
**Description:** of low solubility
**Year:** 1989
**GLP:** no data
**Method:** Generator column technique. The generator column contained 100-120 mesh XAD-2 resin on which the dye had been coated. The resin was coated by adding 0.03 g of dye to several millimeters of acetonitrile in a 50 mL round bottom flask before the addition of 1.3 g of resin.

A 0.25 X 10 stainless steel column fitted with a 0.5 micrometer exit and 1.0 micrometer inlet frit was dry packed with the resin and dye material.

Unbuffered distilled water was pumped through the columns at a flow rate of 0.1 to 2.0 mL per minute. Before taking the first samples, at least one liter of water was pumped through the column.

The concentrator column was a 30 X 0.8cm pyrex tube containing a 5 cm section XAD-2 resin with containment plugs of glass wool on either side. After water had been

allowed to flow through the generator and concentrator columns, the concentrator column was eluted with 2-3 mL of acetonitrile into a tared 20 mL vial.

Quantitation was performed by HPLC using Kratos Model 400 pump acetonitrile/water at a flow rate of 1.3 mL per minute.

**Test substance:** C.I. Disperse Blue 79:1

**Reliability:** (1) valid without restrictions
Meets generally accepted scientific method and is described in sufficient detail.

**Flag:** Critical study for SIDS endpoint

**Reference:** (1)

## ENVIRONMENTAL FATE AND PATHWAYS

### 3.1.1 Photodegradation

**Type:** Air
**INDIRECT PHOTOLYSIS**
**Sensitizer:** OH
**Conc. of sens.:** $1.5 \times 10^{6}$ OH/cm$^3$
**Rate constant:** $226.06 \times 10^{-12}$ cm$^3$/molecule-sec
**Degradation:** 50% after 0.568 hours
**Method:** other (calculated): AOP Program (v1.90)
**GLP:** no
**Test substance:** C.I. Disperse Blue 79:1
**Reliability:** (2) valid with restrictions; accepted calculation method
**Flag:** Critical study for SIDS endpoint
**Reference:** (17)

### 3.1.2 Stability in Water

**Type:** biotic
**$t_{1/2}$ pH 6. 8:** <= 4 hour
**Deg. Product:** yes
**Year:** 1995
**GLP:** no data
**Method:** Disperse Blue 79:1 was reduced in three, high organic carbon content anoxic sediment-water systems.

**Result:** The half-life ranged from 40 minutes to 4 hours. The reaction pathway for the sediment-mediated reduction of Disperse Blue 79:1 resulted principally in the formation of a N,N-disubstituted 1,4-diaminobenzene, 3-bromo-6-nitro-1, 2-diaminobenzene, and a benzimidazole.

**Test substance:** C.I. Disperse Blue 79:1

**Conclusion:** Results of this study suggest that Disperse Blue 79:1 can undergo rapid reductive transformation in anoxic bottom

sediments, resulting in the release of aromatic amines to the water column.

**Reliability:** (2) valid with restrictions
Accepted calculation method.

**Flag:** Critical study for SIDS endpoint

**Reference:** (14)

## 3.3.1 Transport between Environmental Compartments

**Type:** adsorption
**Media:** water - soil
**Year:** 1989
**GLP:** no data
**Method:** Water solubilities and octanol/water partition coefficients were used to predict expected concentration factors for sediment and biota.

**Result:** The results show that Disperse Blue 79:1 has a potential toward sediment sorption and bioconcentration. Measured water solubility was 5.2 ug/l. The calculated LogKp (sediment concentration factor) was 3.9 and the calculated LogBCF (bioconentration factor) was 4.1. The log of the measured partition coefficient (octanol/water) was 4.8.

**Test substance:** C.I. Disperse Blue 79:1

**Conclusion:** Available data from this study suggest that Disperse Blue 79:1 is likely to accumulate extensively in sediment and biota.

**Reliability:** (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment.

**Flag:** Critical study for SIDS endpoint

**Reference:** (15)

## 3.5 Biodegradation

**A. ANAEROBIC DEGRADATION STUDY 1**
**Type:** aerobic and anaerobic
**Inoculum:** activated sludge
**Contact time:** 5 day
**Result:** other: degraded under anaerobic conditions
**Deg. Product:** not measured
**Year:** 1995
**GLP:** no data
**Method:** This study, conducted by EPA, was done to determine the effectiveness of a wastewater treatment plant from a Disperse Blue 79:1 production facility in the removal of dye from a waste stream.

Grab samples were collected from the effluent of a production plant, the bottom of a secondary clarifier and the effluent of a waste treatment plant (WTP) over a period of five days.

**Result:** The highest concentrations of Disperse Blue 79:1 were found in the samples collected from the bottom of the secondary clarifier. Significant reduction (90%) of the dye concentration was observed in the WTP effluent (e.g. 116 mg/kg) compared to the WTP influent (e.g. 1,714 mg/kg) over the five day period.

No degradation of the dye was observed in the waste stream or WTP of the production plant. No Disperse Blue 79:1 was detected in the sediment or water samples downstream of the point where treated effluent enters the river.

**Test substance:** C.I. Disperse Blue 79:1

**Conclusion:** The results suggest that the primary dye removal process occurs in the settling of particulate matter in the primary and secondary clarifiers. Most of the dye is removed from the WTP by the settling of this particulate matter and adsorption on to activated sludge.

Accumulation in the bottom sediments does not occur, as shown in the sediment and water samples taken downstream of the production plant WTP.

Complete removal of Disperse Blue 79:1 from the Plant effluent occurred through degradation under anaerobic conditions.

**Reliability:** (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

**Flag:** Critical study for SIDS endpoint

**Reference:** (8)

**B. ANAEROBIC DEGRADATION STUDY 2**

**Type:** aerobic and anaerobic
**Inoculum:** Activated Sludge from the Milwaukee Metropolitan Sewerage District South Shore Wastewater Treatment Plant
**Concentration:** 443 mg/l related to Test substance
7.86 mg/l related to Test substance
**Contact time:** 15 day
**Degradation:** = 98.2% after 15 day
**Year:** 1989
**GLP:** no data
**Method:** EPA Study; Degradation via anaerobic digester

**Result:** A 98% reduction in the average concentration of dye in the final effluent was observed.

**Test substance:** C.I. Disperse Blue 79:1

**Conclusion:** The majority of the Disperse Blue 79:1 fed to an activated sludge system was removed in the waste activated sludge.

**Reliability:** (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

**Flag:** Critical study for SIDS endpoint

**Reference:** (7)

## ECOTOXICITY ELEMENTS

**Aquatic Organisms**

### 4.1 Acute/Prolonged Toxicity to Fish

See section 4.5.1.

### 4.2 Acute Toxicity to Aquatic Invertebrates

**Species:** Daphnia magna (Crustacea)
**Exposure Period:** 24 hours
**Unit:** mg/l
**Analytical Monitoring:** no data
**EC0:** =1.6
**EC50:** =16
**EC100:** >50
**Method:** OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
**Year:** 1984
**GLP:** yes
**Test Substance:** C.I. Disperse Blue 79 (CAS No. 12239-34-8)
**Remark:** Results are given in mg (active substance)/1.

**Reliability:** (1) valid without restriction
Comparable to guideline study

**Flag:** Critical study for SIDS endpoint

**Reference:** (4)

### 4.3 Toxicity to Aquatic Plants e.g., Algae

**A. BIOMASS**
**Species:** Scenedesmus subspicatus (Algae)
**Endpoint:** biomass
**Exposure Period:** 72 hours
**Unit:** mg/l
**Analytical Monitoring:** no data

**EC10:** = 2.9
**EC50:** = 15
**Method:** OECD Guide-line 201 "Algae, Growth Inhibition Test"
**Year:** 1984
**GLP:** yes
**Test Substance:** C.I. Disperse Blue 79 (CAS No. 12239-34-8)
**Remark:** Results are given in mg (active substance)/l.

**Reliability:** (1) valid without restriction
Comparable to guideline study

**Flag:** Critical study for SIDS endpoint

**Reference:** (4)

B. **GROWTH RATE**
**Species:** Scenedesmus subspicatus (Algae)
**Endpoint:** growth rate
**Exposure Period:** 72 hours
**Unit:** mg/l
**Analytical Monitoring:** no data
**NOEC:** = 2
**EC10:** = 3
**EC50:** = 9.5
**Method:** OECD Guide-line 201 "Algae, Growth Inhibition Test"
**Year:** 1984
**GLP:** yes
**Test Substance:** C.I. Disperse Blue 79 (CAS No. 12239-34-8)
**Remark:** Results are given in mg (active substance)/l.

**Reliability:** (1) valid without restriction
Comparable to guideline study.

**Flag:** Critical study for SIDS endpoint

**Reference:** (4)

## 4.5.1 Acute/Chronic Toxicity to Fish

**Species:** Oncorhynchus mykiss (Fish, fresh water)
**Endpoint:** length, weight, reproduction rate, survival
**Exposure period:** 122 day
**Unit:** µg/l Analytical monitoring: yes
**NOEC:** >= 4.8
**Year:** 1991
**GLP:** yes
**Method:** An early life stage toxicity study of test substance C.I. Disperse Blue 79:1 in Rainbow Trout using a flow-through system was completed in 1991 at ABC Laboratories, Inc., Columbia, MO.

Newly fertilized eggs (fertilized < 4 hours before study initiation) were used for the initiation of the study with exposure continuing for 122 days post-hatch. A 2-liter

proportional diluter system was used to maintain constant test concentrations. Exposure concentrations of test substance were determined by spectrophotometric analysis.

The test system dilution water consisted of deep well water which had been passed through a reverse osmosis system then blended back with additional well water to a total hardness of approximately 160-180 mg/l (as $CaCO_3$)and a pH of approximately 8.3. The water temperature was maintained at 10 +/- 1.5 degrees C during egg incubation and 12 +/- 1.5 degrees C during fry growth. The flow rate was 303 L/day initially and increased to 572 L/day during the final two weeks of the study.

The mean measured concentrations of test substance were 0.36, 0.58, 1.2, 2.5, and 4.8 µg/l. These values ranged from 92% to 116% of the nominal test concentrations of 0.31, 0.63, 1.3, 2.5, and 5.0 µg/l. The high nominal test concentration of 5.0 µg/l was considered to be the limit of solubility for the test substance.

**Result:** The Maximum Acceptable Toxicant Concentration (MATC) Limits, which consists of the no-observed effect concentrations (NOEC) and the lowest observed effect concentration (LOEC), is based on the statistically analyzed parameters of hatchability, survival, and fry growth (length and weight). No statistically significant reductions in hatchability were detected at any test concentration. Fry survival was analyzed at four intervals: 20, 60, 90, 122 days post-hatch. No statistically significant survival reductions were indicated at any test level for either the 20 or 60 day post hatch intervals. Marginally significant reductions in survival were detected at 2.5 µg/l for both the 90 and 122 day post-hatch intervals, but these reductions were not considered to be concentration-related or biologically significant. Therefore, the 2.5 µg/l dose was not considered as an effect level with regard to survival. Length was not significantly reduced at any test level when measured at 60, 90, 122 days post-hatch. At study termination (122 days post-hatch), weight was not significantly reduced at any test level.

**Test Substance:** C.I. Disperse Blue 79:1 Purified Presscake, Lot Number KA0008, BRRC Sample Number 53-10. Green powder at room temperature, >97% pure.

**Conclusion:** Based on the results from this study, the NOEC was determined to be greater than or equal to the highest measured test concentration of 4.8 µg/l. This measured test concentration was based on the highest nominal test level of 5.0 µg/l, which is considered to be the limit of water solubility. No LOEC could be determined because there were no concentration-related effect levels. Therefore, a point estimate MATC value (i.e., the geometric mean of the NOEC and the LOEC) could not be calculated. [ABC Laboratories, Inc., 1991]

The U.S. Environmental Protection Agency has concluded that the ecological risks of C.I. Disperse Blue 79:1 are expected to be low, based on the low toxicity observed at its water solubility (equal to or greater than 4.8 µg/l). [EPA, 1993]

**Reliability:** (1) valid without restriction
Valid without restriction.
Meets national standards method. U.S. E.P.A., 40 CFR 797.1600 Fish Early Life Stage Toxicity Test with Modification.

**Flag:** Critical study for SIDS endpoint

**Reference:** (2)(3)

## HEALTH ELEMENTS

### 5.1.1 Acute Oral Toxicity

**Type:** 14-Day Range Finding for 90-Day Subchronic Toxicity
**Species:** rat
**Strain:** Sprague-Dawley
**Sex:** male/female
**Number of Animals:** 5
**Vehicle:** corn oil
**Value:** = 2500 mg/kg bw
**Year:** 1991
**GLP:** yes
**Method:** Study conducted to comply with GLP regulations, TSCA, and 40 CFR part 793.

Male and female rats (5 per group) were administered the test substance by oral gavage at concentrations of 0, 100, 500, 1000, or 2500 mg/kg/day 5 days per week for 2 weeks plus an additional dose on the following Monday (11 doses).

**Result:** No treatment-related effects on daily clinical signs, body weights, body weight gains, food consumption, and necropsy were observed at any dose level.

**Test substance:** C.I. Disperse Blue 79:1 Purified Presscake, Lot Number KA0008, BRRC Sample Number 53-10. Green powder at room temperature, >97% pure.

**Conclusion:** Based on the lack of evidence of treatment-related effects, it was concluded that a dose of 2500 mg/kg/day is the maximum amount of C.I.Disperse Blue 79:1 that can be administered to rats on a continuous basis.

**Reliability:** (1) valid without restriction
GLP guideline study; 40 CFR Part 793

**Flag:** Critical study for SIDS endpoint

**Reference:** (3) (13)

## 5.4 Repeated Dose Toxicity

**Species:** rat
**Sex:** male/female
**Strain:** Sprague-Dawley
**Route of admin.:** gavage
**Exposure period:** 90 days
**Frequency of treatment:** 5 days per week
**Doses:** 250, 1250, 2500 mg/kg bw/day
**Control Group:** yes, concurrent vehicle
**NOAEL:** >= 2500 mg/kg
**Year:** 1991
**GLP:** yes
**Method:** Dosing suspensions were prepared at concentrations of 5, 125 and 250 mg/ml of corn oil. Corn oil was used in dosing the control animals. Doses were administered as suspensions in corn oil at a volume of 10 ml/kg/day by gavage five days per week over a period of 13 weeks.

Observations and measurements included mortality, clinical signs, body weights, body weight gains, food consumption, ophthalmic examinations, organ weights, hematology, clinical chemistry, gross pathology and histopathology.

After week 13, the rats were anesthetized and sacrificed.

**Result:** Blue coloration of the body and/or tail was observed in some of the animals from all dose groups of male animals and one female from each of the mid and high dose groups. This coloration is not considered biologically significant since the test substance is a dye with an intense blue color. No other treatment-related observations were made for any group treated with Disperse Blue 79:1.

There were no treatment-related differences in food consumption, body weights, ophthalmic examinations, clinical pathology, organ weights, final body weights, necropsy or histopathology observations in those animals treated with Disperse Blue 79:1.

**Test Substance:** C.I. Disperse Blue 79:1 Purified Presscake, Lot Number KA0008, BRRC Sample Number 53-10. Green powder at room temperature, >97% pure.

**Conclusion:** C.I. Disperse Blue 79:1 did not result in any toxicity when administered by gavage to Sprague-Dawley rats 5 days per week for 13 weeks at levels as high as 2500 mg/kg/day. Higher dosing levels were precluded due to limitations of the amount of test substance that could be suspended in corn oil and the amount of corn oil that could be administered to rats in one day.

The blue coloration observed in hair, tails, urine and fecal material of some of the animals can be attributed to the intense blue color of the test substance.

The NOEL for Disperse Blue 79:1 in Sprague Dawley rats under the conditions of this study was at least 2500 mg/kg/day.

**Reliability:** (1) valid without restriction
Meets generally accepted scientific method and is described in sufficient detail.

**Flag:** Critical study for SIDS endpoint

**Reference:** (3) (13)

## 5.5 Genetic Toxicity In Vitro

**A. BACTERIAL TEST**

**Type:** Ames *S. typhimurium* bacterial mutagenicity assay.

**System of testing:** *S. typhimurium* strains TA1537, TA1538, TA98, and TA100

**Concentration:** 1 to 1,000 µg/plate

**Metabolic activation:** With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**

**Results:**

**Genotoxic effects:** Positive

**GLP:** no data

**Method:** The mutagenicity of the test substance was determined using the Ames *S. typhimurium* bacterial mutagenicity assay. Experimental results were evaluated by comparing the number of histidine-independent colonies on treated agar plates with control plates. Mutagenicity was established by demonstration of a mutagenic dose-response relationship.

The test chemical was assayed at a dose range of 1 to 1,000 µg/plate, both with and without metabolic activation, in *S. typhimurium* strains TA 1537, TA1538, TA98, and TA100 obtained from Dr. Bruce Ames of the University of California at Berkeley. Metabolic activation was achieved by an Aroclor 1254-stimulated rat liver system.

The test article was serially diluted in DMSO and added at a volume of 0.05 ml to the plate incorporation assay consisting of 2.00 ml of an agar medium, 0.05 ml of the indicator organisms (about $10^8$ bacteria), and 0.50 ml of the metabolic activation mixture (if appropriate). Plates were incubated for 48 hours at 37° C, after which revertent colonies were counted using a BioTran II automated colony counter when possible or manually with an electric probe colony counter when precipitation precluded automatic counting. All assays were repeated at least once on a separate day.

**Test substance:** Foron Navy SE-2GRL, purity: no data

**Remarks:** Under conditions of the test, it was concluded that the test substance is mutagenic in *S. typhimurium* strains TA1537, TA1538, TA98,and TA100.

**Reliability:** (1) valid without restriction
Meets generally accepted scientific data.

**Flag:** Critical study for SIDS endpoint

**Reference:** (18)

**B. NON-BACTERIAL IN VITRO TEST**

**Type:** Mammalian cell gene mutation assay
**System of testing:** Mammalian cell V79
**Concentration:** Without metabolic activation: 0.05 to 1.0 µg/ml; with 2.5 to 750 µg/ml
**Metabolic activation:** With [ ]; Without [ ]; With and Without **[X]**; No data [ ]
**Results:** NEGATIVE
**GLP:** Yes
**Method:** Other

**Test substance:** C.I. Disperse Blue 79 (CAS 12239-34-8), purity: no data

**Remarks:** The substance was non-genotoxic under conditions of test.

**Reliability:** (1) valid without restriction
Meets generally accepted scientific data.

**Flag:** Critical study for SIDS endpoint

**Reference:** (16)

## 5.6 Genetic Toxicity In Vivo

**A. MICRONUCLEUS ASSAY**

**Type:** Micronucleus assay
**Species/strain:** Mouse/NMRI
**Sex:** Male/Female
**Route of Admin.:** Gavage
**Exposure period:** No data
**Doses:** 5,000 mg/kg
**Results:** No induction of chromosome mutations.
**Genotoxic effects:** Not considered mutagenic
**Method:** OECD Guideline 474: "Genetic Toxicology: Micronucleus Test." (1983)
**GLP:** Yes
**Test substance:** C.I. Disperse Blue 79 (CAS 12239-34-8), purity: no data

**Reliability:** (1) valid without restriction
Meets generally accepted scientific method and is described in sufficient detail.

**Flag:** Critical study for SIDS endpoint

**Reference:** (16)

**B. DROSOPHILA SLRL TEST**

**Type:** Drosophila SLRL test
**Species:** Drosophila melanogaster
**Sex:** male/female
**Strain:** other: Canton-S Wild Type stock
**Route of admin.:** s.c.
**Exposure period:** Administration of the chemical was by injection to 2-3 day old males, who were mated to untreated females. F-1 females were mated individually to brothers. Running time was 19-21 weeks.
**Doses:** 50 ppm
**Result:** negative
**Year:** 1990
**GLP:** yes
**Method:** The chemical C.I. Disperse Blue 79:1 was tested for mutagenic activity (the induction of sex-linked recessive lethal mutations) in Drosophila melanogaster adult males exposed by injection.

The males were injected with approximately 0.3 µl of the test material at a concentration of 50 ppm in 1.9% DMSO and 0.1% Tween 80 carried in 0.7% aqueous saline. This combination of solvents was chosen based on the limited solubility of the test chemical. The material was not toxic at this concentration and no male sterility was induced.

A standard genetic scheme (Basc females crossed with Canton-S wild type males) was employed and post-meiotic germ cells at the time of exposure were tested for lethal mutations.

**Result:** The sex-linked recessive lethal results (shown below) show no difference between treated samples and negative controls. All frequencies are well within the laboratory's range of recent historical control values:

| | | |
|---|---|---|
| DB 79:1, 50 ppm | 17/14740 | (0.115%) |
| negative control | 16/14416 | (0.111%) |
| DMN 500 ppm (positive control) | 58/1208 | (4.801%) |

**Test substance:** C.I. Disperse Blue 79:1 Purified Presscake, Lot Number KA0008, BRRC Sample Number 53-10. Green powder at room temperature, >97% pure.

**Conclusion:** It is concluded that C.I. Disperse Blue 79:1 does not induce mutations in the post-meiotic germ cells of Drosophila melanogaster when administered by injection to adult males.

**Reliability:** (1) valid without restriction
Meets national standards method. EPA OPPTS 870.5275

**Flag:** Critical study for SIDS endpoint

**Reference:** (3)(5)

## 5.9 Developmental Toxicity/Teratogenicity

**A. RAT**

**Species:** rat
**Sex:** female
**Strain:** Sprague-Dawley
**Route of admin.:** gavage
**Exposure period:** Days 6 through 15 of gestation
**Frequency of treatment:** Once daily
**Duration of test:** 20 days
**Doses:** 0, 500, 1000, or 2000 mg/kg/day in corn oil.
**Control Group:** yes, concurrent vehicle
**NOAEL Maternalt.:** >= 2000 mg/kg bw
**NOAEL Teratogen.:** >= 2000 mg/kg bw
**Year:** 1990
**GLP:** yes
**Method:** Pregnant Sprague-Dawley rats were exposed by gavage to C.I. Disperse Blue 79:1 once daily on days 6 through 15 of gestation.

**Result:** At scheduled sacrifice on gestational day 20, maternal body weights and weight gains were equivalent for all groups for all time points. No maternal clinical signs appeared to be treatment related except for green, dark and/or dark green feces in the treated groups. Maternal food consumption showed no treatment related differences and all gestational parameters were equivalent across all groups including pre and postimplantation loss and fetal body weights/litter. There were no treatment related increased incidences in individual or pooled external, visceral, skeletal or total fetal malformations or variations.

**Test substance:** C.I. Disperse Blue 79:1 Purified Presscake, Lot Number KA0008, BRRC Sample Number 53-10. Green powder at room temperature, >97% pure.

**Conclusion:** In conclusion, C.I. Disperse Blue 79:1 administered by gavage during major organogenesis in Sprague-Dawley rats resulted in no maternal or developmental toxicity at any dose tested. The "no observable adverse effect level" (NOAEL) for maternal and developmental toxicity of C.I. Disperse Blue 79:1 in rats is therefore at least 2000 mg/kg/day under the conditions of this study.

**Reliability:** (1) valid without restriction
GLP guideline study.

**Flag:** Critical study for SIDS endpoint

**Reference:** (3)(11)

**B. RABBIT**

**Species:** rabbit
**Sex:** female
**Strain:** New Zealand white
**Route of admin.:** gavage
**Exposure period:** 13 days (gestational days 6-18)
**Frequency of treatment:** once daily
**Duration of test:** 30 days
**Doses:** 0, 100, 300, 600 mg/kg/day
**Control Group:** yes, concurrent vehicle
**NOAEL Maternalt.:** = 100 mg/kg bw
**NOAEL Teratogen.:** = 300 mg/kg bw
**Year:** 1991
**GLP:** yes
**Method:** Artificially inseminated New Zealand White rabbits, 16 females per group, were exposed to test substance by gavage once daily on gestational days 6 through 18 at doses of 0, 100, 300, or 600 mg/kg/day in corn oil. Clinical observations were taken daily and maternal body weights were taken at regular intervals from gestational days 0 through 30.

At scheduled sacrifice on gestational day 30, the does were subjected to a gross necropsy and full examination.

**Result:** Maternal body weights and weight changes were statistically equivalent across all groups, for all intervals evaluated, but maternal gestational weight change was clearly reduced at the 300 and 600 mg/kg/day dose levels. Gravid uterine and liver weights were unaffected by treatment. Food consumption was equivalent across all doses for all intervals except for a significant increase at 600 mg/kg/day for gestational days 6 through 9.

Gestational parameters, including pre- and postimplantaton loss and fetal body weights per litter, were statistically equivalent across all groups. A slight but not statistically significant reduction in fetal body weights per litter (all fetuses and males, but not females) was observed at 600 mg/kg/day, unaccompanied by any other indications of developmental toxicity. Also, no treatment-related increased incidences of individual or pooled external, visceral, skeletal or total fetal malformations or variations were observed.

**Test substance:** C.I. Disperse Blue 79:1 Purified Presscake, Lot Number KA0008, BRRC Sample Number 53-10. Green powder at room temperature, >97% pure.

**Conclusion:** In conclusion, C.I. Disperse Blue 79:1 administered to New Zealand White rabbits by gavage during gestation resulted

in maternal toxicity at 300 and 600 mg/kg/day and a slight reduction in fetal body weight at 600 mg/kg/day.

There was no evidence of teratogenicity at any dose tested.

Therefore, the NOAEL for maternal toxicity was 100 mg/kg/day and for developmental toxicity was 300 mg/kg/day under the conditions of this study.

**Reliability:** (1) valid without restriction
GLP guideline study.

**Flag:** Critical study for SIDS endpoint

**Reference:** (3)(12)

## 5.10 Other Relevant Information

**Type:** Metabolism
**Species:** Rat
**Year:** 1991
**GLP:** Yes
**Method:** Six rats per dose level were randomly selected and placed individually into Roth-type metabolism cages for an acclimation and fasting period of 15 hours prior to dose administration. The test substance in corn oil was administered to four male and four female rats per group. The target concentrations were 500 and 50 mg/kg for the high and low dose groups, respectively. The target dose volume was 4 ml/kg of body weight and a target radioactivity of 10-15 uCi was given to each animal.

Urine and feces were collected at 6, 12, 24, 48, 72 and 96 hr post-dosing. Room air was drawn through Roth-type metabolism cages, specifically designed for collection, at a rate of approximately 500 ml/min. Expired 14-CO2 was trapped at 12, 24, 48, 72 and 96 hours post-dosing.

Ninety-six hours after administration of the dose, the animals were anesthetized and sacrificed. Selected organs were collected for analysis.

Analysis of dosing suspensions for Disperse Blue 79:1 was conducted prior to and at the conclusion of the definitive study. Analysis of urine and feces for Disperse Blue 79 and the suspected metabolite, BDNA, was also conducted using HPLC.

**Result:** The overall recovery for the high dose was 98.0 +/- 2.1% for the males and 92.5 +/- 2.6% for the females. For the low dose, overall recovery was 94.0 +/- 3.4% for the males and 91.7 +/- 3.3% for the females.

The majority of the radioactive dose (greater than 73%) was excreted in the feces within the first 24 hours and an additional 5-12% excreted in the second 24 hour period.

Excretion of radioactivity was virtually complete by 48 hours post-dosing with less than 1% of the fecal radioactivity excreted between 48 and 96 hours.

Approximately 6% of the administered dose was excreted in urine during the 96 hour collection period, with the majority (almost 5%) excreted during the first 24 hours. Minor amounts of radioactivity were also recovered in expired carbon dioxide (0.02-0.10%), the tissues (0.03-0.14%), and in the carcas/pelt (0.00-1.40%)

Analysis of individual feces samples collected from each sex in each dose group demonstrated that the majority of detectable radioactivity was unchanged C-14 labeled DB 79:1.

An unresolved metabolite peak accounted for the balance of the radioactivity in the feces samples from all dose levels. Analysis of pooled urine samples collected at 12, 24 and 48 hour post-dosing, from each sex in each dose group, showed an unidentified metabolite effectively accounting for all of the detectable radioactivity in the urine samples from all dose levels. The suspected metabolite, 6-bromo-2,4-dinitroaniline(BDNA), did not appear in any of the urine or feces samples.

**Test substance:** C.I. Disperse Blue 79:1 Purified Presscake, Lot Number KA0008, BRRC Sample Number 53-10. Green powder at room temperature, >97% pure.

**Conclusion:** The recovery of 85 to 91% of the administered dose in the feces alone indicates that DB79:1 is probably not extensively absorbed from the GI tract of the rat following oral ingestion. It was therefore concluded by the investigators that DB79:1 is substantially cleared from the GI tract following oral doses and does not appear to be extensively metabolized.

**Reliability:** (1) valid without restriction
Meets generally accepted scientific method and is described in sufficient detail.

**Flag:** Critical study for SIDS endpoint

**Reference:** (3)(6)

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